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Base-Line Growth Studies of 'First Lady' Marigold in Controlled Environments

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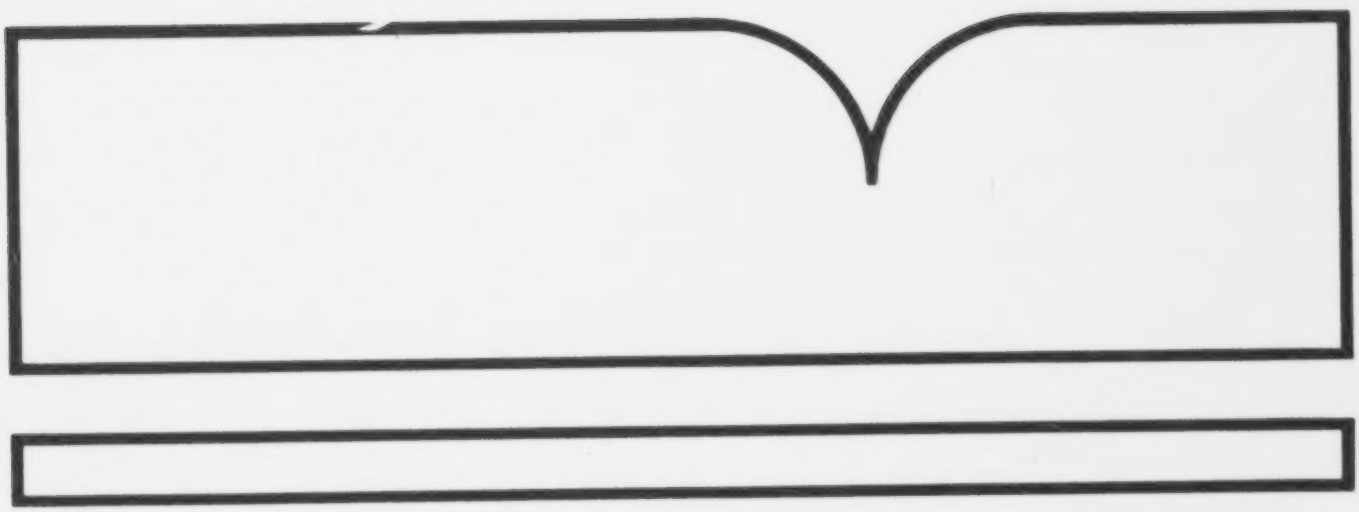

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16. ABSTRACT

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Base-line Growth Studies of 'First Lady' Marigold in Controlled Environments[1]

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Abstract. **'First Lady' marigold (Tagetes erecta L.) was grown 3 times in each of 5 different controlled environment facilities to establish baseline growth rates for this species. Plants were grown under a standardized set of environmental conditions established with a common set of standardized instruments. Data on plants grown only once in two additional laboratories are reported for comparative purposes. The variation in growth within each laboratory was greater than the average variation among laboratories for cotyledon length and width, plant weight measurements, and 3rd leaf length but not for leaf width. The base-line growth data, based on the described procedures, can be used to compare marigold growth in other controlled environments and to provide a check on the operational characteristics of research facilities.**

There is need for improved comparability among experiments conducted in controlled environments by researchers at different locations (4). The Committee on Growth Chamber Environments of the American Society for Horticultural Science has undertaken cooperative studies designed to increase uniformity in growth chamber research (9). Plants of different species were grown under a standard set of environmental conditions with growing and environmental measurement procedures standardized to minimize variability (13, 14) and inadvertent stress (10). A set of environmental measuring instruments was circulated among laboratories for each study conducted. Baseline growth data have been reported for 'Grand Rapids' lettuce in a previous report (2).

Previous studies with marigold have demonstrated its extreme sensitivity to environmental conditions (1, 5, 6, 7, 8, 11, 12, 15). In these studies, 'First Lady' marigold was grown under a uniform set of environmental conditions 3 separate times at each of 5 locations and once at each of 3 locations to establish base-line growth rates that can be used to compare marigold growth in different growth chambers and serve as a check on operation and environmental control in plant growth chambers. The environmental conditions that were duplicated in each laboratory were set to produce normal plant growth and not necessarily to provide optimum growing conditions.

Methods

'First Lady' marigold seed from a single lot was separated with an air stream to obtain a quantity of seed of uniform weight, stored at one location, and distributed in moisture-proof containers to each experimenter. Seed was stored in a double plastic bag or sealed container at 5°C.

White round standard plastic pots (12.7 cm diameter) with 4 drainage holes in the bottom were used. A volume of potting medium equivalent to 24 loosely filled pots of commercial peat-lite medium (1 part sphagnum peatmoss to 1 part vermiculite by volume with added nutrients) was moistened with 6 liters of distilled water and mixed thoroughly. Twenty pots were filled level without packing, and then lightly tapped to settle the peat-lite 1.5 cm. They were refilled to the top with additional mix and leveled.

A modified Hoagland solution (3) was applied to pots with the automatic watering system as described previously (2). Immediately after the pots were filled with medium and placed in the growth chamber, the pump was allowed to run until the medium was saturated and drainage was observed. The timer was set so watering occurred every 4 hr (6 times a day) with 50 ml of solution applied per pot at a rate that did not exceed 50 ml per min. In order to equalize and stabilize the nutrient content, the medium was watered with timed additions for 2 days before the marigold was seeded. On day 21, the watering rate was doubled by placing 2 watering tubes in each container.

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[8]Appreciation is extended to V. P. Bonaminio (NC State Univ.), H. Hellmers (Duke Univ.) and T. Matsui (Biotron Inst., Fukuoka, Japan) for their participation in these studies, to R. J. Gladon, R. Hodgson, and R. A. Norton for critically reading the manuscript, to Geo. J. Ball Inc., West Chicago, IL, for providing the seed used in these studies, and to W. R. Grace and Co., Travelers Rest, SC for providing the peat-lite medium.

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The watering provided an excess for drainage through the pots. The first irrigation coincided with the start of the light period.

Six marigold seeds were sown 1.5 cm apart on the surface of the center of each pot 2 hr after the start of the light period. The seeds were covered with a thin layer of peat-lite medium ground through a fine mesh screen. This covering was moistened with distilled water using an atomizer.

The pots were randomly numbered in the growth chamber in 4 rows of 5 pots each with edges touching during the first week. On day 7, the modal cotyledon length of plants whose cotyledons were 10 mm long or longer was determined. A plant with near modal cotyledon length and with uniform true leaves was selected in each pot. The extra plants were removed by clipping at the medium surface. Five randomly selected plants were harvested at 7-day (to the hour) intervals after seeding for 4 weeks. After each harvest, the remaining pots were randomized and spaced uniformly over the study area (i.e. during week 2, pots were 2 cm apart; during week 3, pots were 7.5 cm apart, and during week 4, pots were 10 × 20 cm apart).

Data recorded at each harvest included number of nodes with leaves over 1 cm, length and width of the longest cotyledon, length and width of the 3rd leaf, length of the stem from the cotyledonary node to the top of the leaf node with a leaf over 1 cm long, and fresh and dry weight of the shoot above and including the cotyledonary node. For dry weight determinations, plant tissue was dried at 80°C for 2 days and then placed in a desiccator and allowed to come to room temperature before weighing.

Studies were conducted in 7 laboratories in growth chambers (Table 1) all containing cool white fluorescent and incandescent lamps to provide about 325 $\mu E\ m^{-2}s^{-1}$ photosynthetically active radiation (PAR) measured with a Lambda quantum sensor (sensitivity between 400-700 nm) and about 0.08 $W\ m^{-2}nm^{-1}$ of radiation between 720-820 nm measured with a Lambda far-red (FR) sensor. All laboratories used the same instrument package for calibration of local sensors and start-up of each study. The daylength was 16 hr. Temperature control equipment was calibrated at the start of the experiment to provide air temperatures of 25°C (day) and 20°C (night) as measured with a shielded, but not aspirated, sensor placed 1 cm above the center container of the experiment. Relative humidity was controlled in chambers at 70% day and night where possible, Ambient CO_2 level was used with make up air provided in all chambers. Environmental parameters as measured in the different studies are given in Table 2.

Three studies were randomly conducted at various times in laboratories I-V while one study each was conducted in laboratories VI-VII. The data from laboratories I-V were subjected to analysis of variance with laboratory and time of harvest as fixed effects and studies as a random effect. Logarithmic transformations were used to stabilize the variance for fresh weight, dry weight, and stem length. Square root transformation was used to stabilize the variance for number of nodes. Transformations were not used on the length and width of the cotyledons and 3rd leaf because no improvements in the variance stability were found in the earlier studies with lettuce (2). Length and width of the cotyledon were compared for only the first 3 weeks because in most studies the cotyledons were senescing or partially necrotic by the final harvest. Stem length and number of nodes/leaf were not of sufficient magnitude at the 1st harvest, and thus were only compared over the last 3 harvests. Length and width of the 3rd leaf were compared over the last 2 harvests.

Results

The mean values for growth of marigold plants in successive studies revealed considerable variation as indicated by the range of minimum and maximum values at 28 days for locations I-V (Table 3). There was significant variation among laboratories only in cotyledon width, node number, and width of 3rd leaf. The variation in growth among laboratories was generally less than the variation obtained for repeated studies within laboratories (Table 4). Only the variation in cotyledon width,

Table 1. Chamber specifications in the different laboratories.

Laboratory	Chamber manufacturer and type	Barrier[Y] material	Reflective specifications of surfaces
I Dept. of Horticulture Lafayette, IN	Sherer-Gillett, CEL 512-37, Reach-in	None	White enamel
II Post Laboratory Ithaca, NY	Environmental Growth Chamber Walk-in	Plexiglass	Aluminized Mylar
III EPA Laboratory Las Vegas, NV	Environmental Growth Chamber, M-3, Reach-in	Glass	Polished stainless steel
IV Dept. of Horticultural Sci., Guelph, Ontario	Controlled Environments EY15, Reach-in	None	White enamel
V Plant Stress Laboratory, Beltsville, MD	Environmental Growth Chamber, M-13	Plexiglass	Polished stainless steel
VI Biotron Inst., Kyushi Univ., Fukuoka, Japan	Environment Growth Cabinet KG208SHL	Plexiglass	Polished stainless steel
VII Phytotron, Duke Univ., Durham, NC	Environmental Growth Chamber, M-3, Reach-in	Plexiglass	Specular aluminum

[Y]Transparent barrier between lamps and plant chamber, if any.

Table 2. Environmental parameters measured in the different studies. Laboratories are listed in Table 1.

Laboratory	Study	Radiation PAR[z] (μE m^{-2}s^{-1})	Radiation FR[y] (Wm^{-2}nm^{-1})	Radiation Total[y] at 280-2800 nm (Wm^{-2})	Temperature (°C) Air[x] day	Air[x] night	Medium (day)[w] 1 cm depth	Medium (day)[w] 6 cm depth	Dew point[x] day	Dew point[x] night
I	1	325-340	0.086	142	24.4-26.3	---	23.0-24.6	23.4-24.0	---	---
	2	340	---	--	23.5-26.1	---	---	---	15.6-17.8	---
	3	314-345	0.084-0.090	132	24.5-26.3	19.7-20.6	25.5-26.2	26.3-27.2	17.8-19.8	20.6-23.3
II	1	320-330	---	142.5	25.0	---	---	---	17.5-18.5	12.5-13.5
	2	320-330	---	137.6	25.0	---	---	---	18.5-19.5	12.5-13.5
	3	320-335	0.074-0.079	124.8	25.0-25.5	---	24.5-25.7	24.2-25.5	18.3-20.0	18.3-20.0
III	1	300-340	---	167.1	22.4-24.8	---	21.0-22.4	22.8-23.8	---	---
	2	305-338	0.068-0.079	--	---	---	25.0	23.4	---	---
	3	302-340	0.071-0.082	--	24.8-25.0	20.0-20.2	24.2	23.0	---	---
IV	1	306-328	0.051	96.3	24.2-26.0	18.5-21.0	23.5-24.4	22.2-23.6	17.8-21.4	19.7-21.4
	2	302-328	0.087	162.2	24.2-26.2	17.5-21.0	23.8-24.6	22.2-23.0	17.8-21.4	18.9-21.4
	3	305-325	0.081	157.3	24.0-25.5	20.0-21.0	21.7-23.0	20.5-21.3	18.9-21.1	18.3-21.4
V	1	295-320	0.063-0.075	145.5	24.7-26.9	17.3-21.0	21.1-27.4	26.2-26.7	14.1-22.2	18.3-20.6
	2	290-335	0.063-0.073	147.4	25.1-27.0	19.0-21.0	---	---	12.8-18.8	18.3-22.8
	3	285-330	0.068-0.079	154.3	25.0-27.0	18.0-20.3	---	---	16.1-18.9	16.1-20.6
VI		302-335	---	--	26.0	19.6	25.6-27.6	24.8-26.3	18.9-19.7	18.9-19.7
VII		325-355	0.119-0.132	210.4	23.7-25.0	20.0	25.6-26.3	25.5-26.5	17.3	21.0

[z]Range over the growing area at the start of the study.

[y]Measured over the center container at the start of the study.

[x]Range of average daily temperature during the study.

[w]Range between the containers at the start of the study.

Table 3. Minimum, maximum and mean values of growth parameters of base-line marigold growth for laboratories I-V.

Growth parameter	Days 7	Days 14	Days 21	Days 28	Min and max values 28 days after seeding[x]
Cotyledon length (mm)	20.7	32.6	32.6	–	(29-36)[y]
Cotyledon width (mm)	6.8	8.4	8.4	–	(7-10)[y]
No. of nodes with leaf 1 cm long	–	2.4	4.9	7.2	5-9
Length of the 3rd leaf (mm)	–	–	106.5	184.3	153-213
Width of the 3rd leaf (mm)	–	–	79.9	138.5	109-161
Stem length[z] (mm)	–	12.2	45.1	93.5	76-111
Fresh wt (g)	0.083	1.012	7.925	40.478	29.2-55.6
Dry wt (g)	0.0069	0.0934	0.7249	3.6091	2.9-5.0

[z]From cotyledonary node to top node with leaf over 1 cm long.

[y]At 21 days from seeding.

[x]Means of the 5 plants in each study.

Table 4. Results of analysis of variance procedures on all the plant parameters measured for laboratories I-V.

Source of variance	Significance Cotyledon Length	Cotyledon Width	ℓn fresh wt	ℓn dry wt	ℓn stem length	$\sqrt{\text{No. of nodes}}$	Length of 3rd leaf	Width of 3rd leaf
Among labs	NS	*	NS	NS	NS	*	NS	**
Studies within labs	***	***	***	***	***	***	***	NS
Harvest	***	***	***	***	***	***	***	***
Lab X harvest	***	NS	NS	NS	NS	NS	*	***
Study X harvest within labs	NS	***	NS	NS	NS	***	***	NS

*, **, ***, [NS]Significant at 5% (*), 1% (**), or 0.1% (***) level or nonsignificant (NS) at 5% level, respectively.

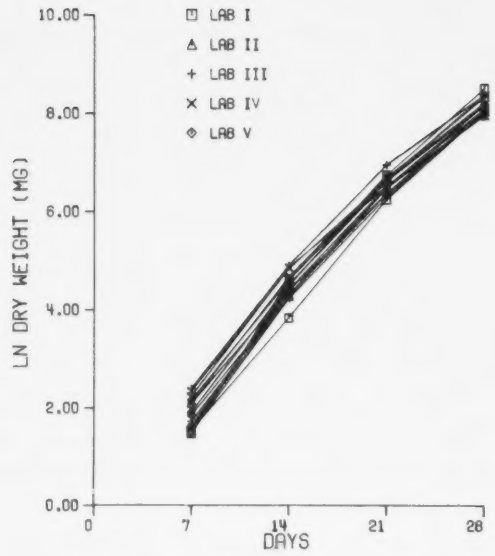

Fig. 1. Mean ℓn dry wt (mg) for each of 3 studies in laboratories I-V plotted for 7, 14, 21, and 28 days after seeding; means of 5 plants.

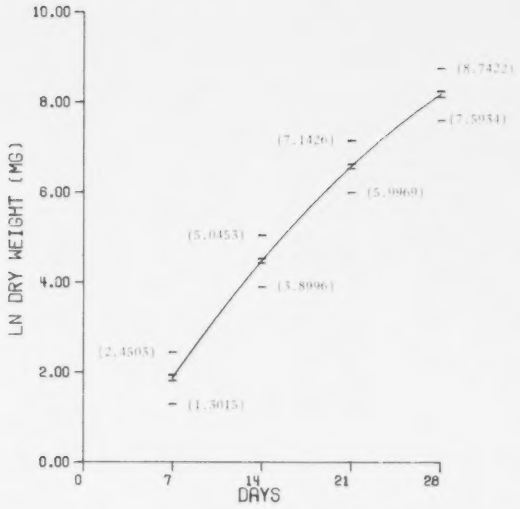

Fig. 3. Plot of $Y = 1.219 + (0.477)\ X - (5.094 \times 10^{-3})\ X^2$. (Y = ℓn dry wt (mg); X – days from seeding). $R^2 = 0.985$. Inner-lines are 95% confidence limits and outer-lines are 95% prediction limits. The numbers in parenthesis are the 95% prediction limits at 7, 14, 21, and 28 days after seeding.

node number and 3rd leaf width was significant among laboratories and all growth factors had significant variation for studies within laboratories except the variation in 3rd leaf width. There was significant interaction of laboratory and harvest only in measurements of cotyledon length and length and width of the 3rd leaf. In contrast, interaction of studies with harvests within laboratories was significant for cotyledon width, number of nodes, and length of 3rd leaf.

Plotting natural logarithm (ℓn) fresh weight, ℓn dry weight, and ℓn stem length versus days after seeding for the mean of laboratories I-V, indicated there was some random crossing of the plots among laboratories. However, these graphs indicate that growth in any one laboratory did not deviate substantially from growth in the others, as was also reported for lettuce (2).

The plots of ℓn dry weight and ℓn stem length for each study in each of laboratories I-V (Fig. 1 and 2) demonstrate that the variation within each laboratory was greater than the average variation among laboratories. There were no significant interactions of studies within laboratories for these parameters (Table 4) as corroborated by the plots.

On the assumption that the variation observed for studies both within each laboratory and among laboratories can be an estimate of the variation to be expected in growth chamber studies with marigold, regression analysis was used to fit a quadratic equation for ℓn dry weight with time after seeding

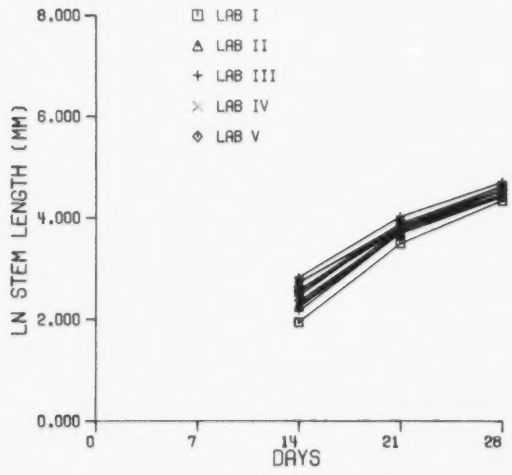

Fig. 2. Mean ℓn stem length (mm) for each of 3 studies in laboratories I-V plotted for 14, 21, and 28 days after seeding; means of 5 plants.

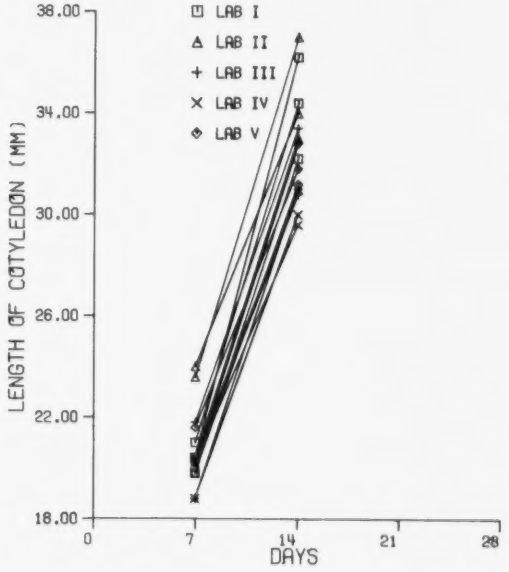

Fig. 4. Mean length of the cotyledon (mm) for 3 studies in laboratories I-V plotted for 7 and 14 days after seeding; means of 5 plants.

Table 5. Number of nodes and mean square root of number of nodes of marigold for laboratoties I-V averaged over the values obtained at the 14, 21, and 28 day harvest.

Laboratory[z]	No. of nodes[y]	$\sqrt{\text{No. of nodes}^{y}}$
I	5.3	2.2 a[x]
II	3.9	1.9 b
III	5.0	2.2 a
IV	4.7	2.1 ab
V	5.2	2.2 a

[z]As listed in Table.

[y]Mean of 3 wk x 3 studies x 5 plants.

[x]Mean separation by HSD, 5% level.

Table 6. Mean length and width of the 3rd leaf of marigold for laboratories I-V averaged over the values obtained at the 14, 21, and 28 day harvest.

Laboratory[z]	Length of 3rd leaf[y] (mm)	Width of 3rd leaf[y] (mm)
I	149.6	115.6 a[x]
II	143.7	101.5 b
III	140.6	107.4 b
IV	139.7	104.0 b
V	153.5	117.4 a

[z]As listed in Table.

[y]Mean of 3 wk x 3 studies x 5 plants.

[x]Mean separation by HSD, 5% level.

(Fig. 3). As for the lettuce studies (2), this is a plot of the regression curve of ℓn dry weight with 95% confidence limits for the average of the marigold studies conducted in laboratories I-V and 95% prediction limits for separate studies that might be conducted by anyone.

The plot of length of cotyledon for each study in each laboratory illustrates the differences observed within laboratories (Fig. 4). The over all effect of laboratories was not significant, but the nature of the separate interactions of laboratory and harvest is illustrated in Fig. 3. There was less variation in this parameter than in the lettuce study (2), but, nevertheless, there was apparently considerable diversity associated with cotyledon response to environmental conditions.

The square root of number of nodes was significantly different among laboratories, but differences were small (Table 5) as in lettuce (2). The variation in number of nodes on marigold plants was greater than that observed in lettuce, with laboratory II having the smallest node number as was found for lettuce growth in this laboratory.

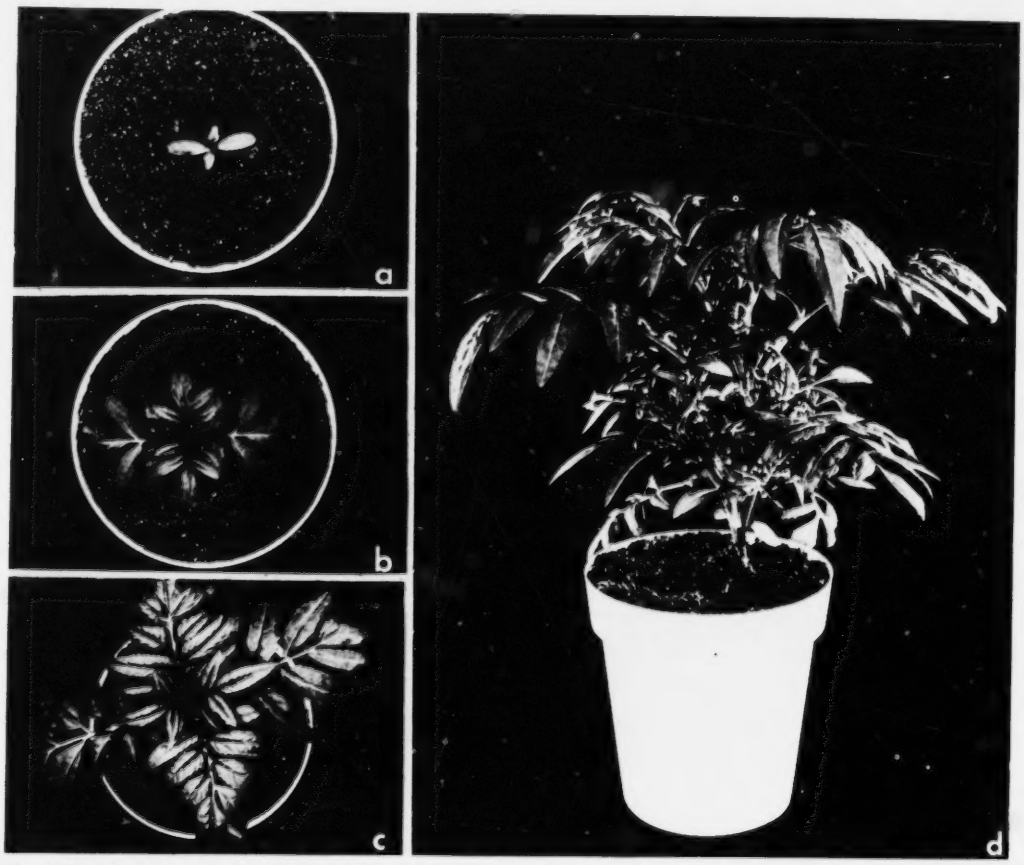

Fig. 5. Visual appearance of representative marigold plants at 7 (a), 14 (b), 21 (c), and 28 (d) days after seeding. Magnifications are 0.3X, 0.3X, 0.26X and 0.30X respectively.

Table 7. Mean relative growth rate (MRGR) of marigold plants at 7-14, 14-21, and 21-28 days after seeding. Each value of MRGR represents the mean of 5 plants. The wt at each date was obtained from separate plants because destructive sampling was used at each harvest.

Laboratory[y]	Study	Mean relative growth rate[z] 7-14	14-21	21-28
I	1	0.34	0.33	0.25
	2	0.45	0.30	0.25
	3	0.42	0.31	0.23
	Lab avg	0.40 ± 0.06[x]	0.31 ± 0.01	0.24 ± 0.01
II	1	0.33	0.30	0.22
	2	0.35	0.29	0.22
	3	0.28	0.31	0.21
	Lab avg	0.35 ± 0.02	0.30 ± 0.01	0.22 ± 0.005
III	1	0.36	0.29	0.20
	2	0.39	0.29	0.26
	3	0.36	0.29	0.23
	Lab avg	0.37 ± 0.02	0.29 ± 0.001	0.23 ± 0.03
IV	1	0.36	0.27	0.24
	2	0.38	0.24	0.21
	3	0.37	0.29	0.21
	Lab avg	0.37 ± 0.01	0.27 ± 0.02	0.22 ± 0.02
V	1	0.37	0.30	0.22
	2	0.35	0.31	0.23
	3	0.39	0.29	0.23
	Lab avg	0.37 ± 0.02	0.30 ± 0.01	0.23 ± 0.005
	Overall avg	0.37	0.29	0.22

[z]Mean relative growth rate = ℓn dry wt $(mg)_2$ − ℓn dry wt $(mg)_1$/$time_2$ − $time_1$.

[y]As listed in Table 1.

[x]SD.

The width of the 3rd leaf, but not the length, was significantly different among laboratories while length, but not width, was significantly different within laboratories (Table 6). The width of leaves was significantly greater in studies conducted in two of the laboratories than in the other three laboratories. This variation among laboratories may have been due to variation in the method used to measure leaf width, for measurement of leaf dimensions is difficult to standardize, as was indicated previously for lettuce leaves (2).

Mean relative growth rates (MRGR) had similar patterns fo. the 3 growth periods of 7-14, 14-21, and 21-28 days over each study and in each laboratory (Table 7). The statistical analysis demonstrated greater differences for studies within, than among laboratories, for these growth parameters as well (Table 4).

At the 2 laboratories (VI-VII) conducting only 1 study (Table 8), the values for all parameters were within the ranges found at the locations with replicated studies (Table 3).

Discussion

These marigold growth studies in combination with the lettuce studies reported previously (2), document the amount of variation in plant growth that researchers can anticipate in separate studies. Less variability will be associated with growth measurements of tissues that have attained finite dimensions at the time of measurement (leaf length and width), than with such factors as plant weight, that are dynamic measurements and will have much greater variability at any particular time of measurement duing plant growth. Although measurements of plant weight at any particular time varied greatly between studies, the rate of growth as compared through calculation of MRGR, was not as variable and MRGR values were similar over all studies. This suggests that MRGR may be the most useful parameter for comparing plant growth in different experiments. Studies conducted randomly over time will have differences in growth that should be recognized by experimenters who use growth chambers. There was little correlation between growth measurements at 7 and 28 days in an individual study, indicating that conditions during seed germination and seedling establishment were not the major controlling factors for the total growth that was measured at 28 days.

As in the lettuce studies, the variation among studies within the same laboratories can not be readily explained. However, there are many factors that were not uniform in the successive studies, and could have affected the marigold growth significantly. Small differences in cotyledon size and hence initial supply of food reserves may have accounted for some of the variation. Other sources of variation may have been slight differences in atmospheric moisture (6), total radiation (6, 14) and spectral (14). Marigold plants are one of the most sensitive species to ethylene contamination, responding to concentrations in the external atmosphere as low as 0.17 $\mu\ell\ \ell^{-1}$ (1, 8). Differences in concentrations of other air contaminants and in concentrations of ambient CO_2 in the make up air, due to

Table 8. Mean values of growth parameters of base-line marigold growth at laboratories VI-VII.

Growth parameter	Days from seeding 7 VI	7 VII	14 VI	14 VII	21 VI	21 VII	28 VI	28 VII
Cotyledon length (mm)	22.0	18.6	30.2	30.8	34.0	33.0	–	–
Cotyledon width (mm)	7.0	7.2	8.4	8.2	8.4	7.8	–	–
No. of nodes with leaf 1 cm long	–	–	3.0	2.8	5.0	4.6	8.0	6.0
Length of the 3rd leaf (mm)	–	–	15	44	114	105	201	200
Width of the 3rd leaf (mm)	–	–	2	30	70	92	134	148
Stem length[z] (mm)	–	–	12.8	18.4	43.4	57.8	85.6	115.6
Fresh wt (g)	0.101	0.073	1.096	1.004	9.316	8.674	38.934	45.855
Dry wt (g)	0.0061	0.0064	0.0931	0.0965	0.7320	0.7777	3.5580	3.8808

[z]From cotyledonary node to top node with leaf over 1 cm long.

variable numbers of people, vehicles, and other plants in the vicinity, may also have been sources of variation. There was better uniformity in growth among the marigold plants in these studies, than among the lettuce plants in previous studies. This may be due to differences in the genetic variation between the two species, or to more uniform standardization of procedures in the marigold studies than in the lettuce studies.

These standardized growth data for marigold along with the growth data for lettuce (2) provide a base-line for investigations to evaluate growth chambers for unexpected factors that may be stimulating or suppressing plant growth. Marigold plants should be useful because: they take little space; they grow rapidly so that evaluations can be completed quickly; they have substantial stem elongation for evaluation of hormonal imbalances; and they develop side branches in response to increasing photosynthetic activity. Investigators are encouraged to utilize these procedures with the objective of improving the credibility and usefulness of controlled environment research in horticulture.

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